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14. ABSTRACT

We hypothesize that the biomechanical perturbations of the brain that yield blast-induced mTBI in injured warfighters can be recreated with reasonable fidelity in rats under carefully controlled experimental conditions, and that several of the characteristic sequelae of blast-induced mTBI observed clinically can be reproduced in a rodent injury model. In many, if not most circumstances yielding blast mTBI, brain injury results from a combination of blast overpressure (BOP) (i.e. primary blast) and head acceleration and/or impact (i.e. tertiary blast). The mTBI resulting from these combined insults may be fundamentally different from that seen from either insult alone.

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INTRODUCTION:

Many warfighters who sustain blast-induced TBI in combat are exposed to a brain insult resulting from a combination of both a shock wave and biomechanical perturbation related to rapid acceleration and/or impact with a solid object (MacDonald et al., 2011). The TBI resulting from these combined insults is likely to be fundamentally different from that seen from either insult alone. We hypothesize that the combined biomechanical perturbations of the brain that yield blast-induced mild TBI in injured warfighters can be recreated with reasonable fidelity in rats under carefully controlled experimental conditions, and that several of the characteristic sequelae of blast-induced mild TBI observed clinically can be reproduced in an established rodent injury model. We anticipate that this model can provide a valuable experimental tool to assist ongoing efforts to mitigate the risks and consequences of blast-induced mTBI in warfighters.

BODY:

Research accomplishments associated with each task outlined in the approved Statement of Work are described below.

Task 1: Manipulate and monitor blast exposure conditions (i.e. incident flow conditions) in the compression-driven shock tube and recreate with reasonable fidelity the biomechanical loading conditions estimated to underlie primary blast-induced mild TBI in warfighters. Establish a mild injury severity based upon loss of consciousness (LOC), histopathology, and neurological and neurobehavioral outcomes.

Using a custom designed, instrumented rat holder for the compression-driven shock tube (fig 1 in Supporting Data section), we have monitored and documented blast exposure conditions associated with varied neurobehavioral disruptions and histopathological outcomes. Neurobehavioral disruptions have been evaluated using spatial navigation in the Morris water maze and the Barnes maze, rotarod performance, ambulation/balance on a rotating pole fig 3 in the Supporting Data section), along with open field and visual and olfactory discrimination assessments. We have examined neurobehavioral and histopathological consequences of blast overpressure (BOP) exposures with peak pressures ranging from 11 to 21 psi, seeking to establish survivable injury conditions yielding reproducibly robust functional impairments. Despite modifications to make the tests more challenging (and more sensitive), performance in the Morris water maze and Barnes maze was not appreciably altered by any of the survivable blast exposure severities. Although impairments were indiscernible with 11 psi peak overpressures, rotarod performance and ambulation/balance on a rotating pole have been clearly disrupted with greater injury severities (14-21 psi peak overpressures) and have also been associated with fairly modest neuropathological changes, which are most pronounced and consistently observed as fiber degeneration in cerebellum, optic tract, corpus callosum, and internal and external capsules. LOC as estimated by prolonged righting times is seen after BOP exposures has been monitored throughout these experiments and although variable, is clearly prolonged in injured rats.

In general, through a series of minor modifications in how the experimental subjects are positioned and secured within the shock tube, we have established exposure conditions that are reasonably controlled, consistent, and biomechanically characterized. Notably, despite being tightly secured in a coarse mesh netting, using high speed videography and accelerometer recordings, we discovered that rats nevertheless experience appreciable acceleration during BOP exposure (fig 1 in

Supporting Data section), and speculate that it is a significant component of the biomechanical factors yielding brain injury under these exposure conditions. We are now developing and implementing measures to systematically manipulate acceleration and displacement as a controllable exposure condition that appreciably contributes to the BOP-induced biomechanical insult to the brain. Regardless of these exposure refinements and the possible role of acceleration, it is clear from this work that brain injuries resulting from shock tube BOP exposures alone are typically mild.

Task 2: Establish conditions yielding a mild injury severity with a surgery-free adaptation of the weight drop brain injury model (or alternative) to create tertiary blast brain injury based upon LOC, histopathology, and neurological and neurobehavioral outcomes.

Three approaches to weight drop brain injuries were evaluated and compared during this reporting period. Using a traditional weight drop device and procedure, in which stainless steel discs are affixed to the rats' skulls and impacted by weights dropped through a cylinder, we initially conducted experiments to determine the optimal height-weight combinations to generate survivable mild TBI and to also evaluate and compare the brain injuries and functional disruptions produced by weight drop with and without accompanying BOP exposure (task 3). Brain injuries produced by a 500 g weight dropped from heights ranging from 1 to 2 m were assessed. The resulting neurobehavioral changes were generally severity-dependent and were accompanied by anticipated histopathological and neurochemical changes (figs 4-8 in Supporting Data section). In particular, histopathological features of weight drop injuries were somewhat different than those seen after BOP, and included greater levels of injury in the cerebral cortex. Initial neurochemical measurements revealed significant elevations in reactive oxygen species and DNA fragmentation at 24 hrs postinjury. LOC as estimated by prolonged righting times was seen after weight drop, although severity-dependent LOC was not clear-cut in these initial evaluations. Parenthetically, since weight drop is combined with BOP exposure in task 3, and larger (i.e. > 400g) rats appear to be more resistant to BOP-induced TBI than are smaller experimental subjects, we chose to work with 300-325 g rats in task 2 as well. We discovered that weight drop-induced skull fractures and mortality were much more common with the smaller sized rats we have used than have been reported with older heavier rats with thicker skulls (400-450 g) that have typically been used by Marmarou and others for weight drop TBI evaluations. Despite this challenge, we believe that we have established survivable weight drop conditions that can be applied to combined injuries in task 3.

For the second approach to weight drop injuries, a weight drop device and instrumented removable helmet were designed to produce weight drop injury without the need to affix a disc to the rat's skull, and are therefore compatible with more closely timed airblast and weight drop exposures. Aluminum helmets were placed over the rats' heads immediately after removal from the shock tube post-BOP and an impact acceleration brain insult was generated within 45 sec of blast exposure using the device pictured in fig 2 in the Supporting Data section. Range-finding experiments were again conducted using varied weight drop severities alone and in combination with BOP exposure (task 3). Despite the advantages of the surgery-free experimental approach, we were unable to produce consistent weight drop injures yielding persistent neurobehavioral deficits due in part we suspect to the mass of the weight drop helmet, which appears to appreciably diminish impact acceleration brain injury from that produced by impact directed to the disc on the exposed skull. Binding of the

dropped weight was also problematic, and design changes were judged to be necessary before proceeding further with this approach.

Our third approach combined elements of the first two approaches. Specifically, a 500 g weight was dropped through a cylinder onto a stainless steel disc that was positioned on the rat's head immediately upon removal from the shock tube using a light weight Mylar head piece. As with the second approach, the scalp is intact and weight drop is performed within 45 sec of blast exposure. Although for identical height-weight combinations the resulting weight drop injury is less severe than seen with approach 1, presumably due to the disc resting on the scalp rather than being directly secured to the skull, recalibrated weight-drop injuries can nevertheless be consistently produced and combined with BOP to examine potential interactions (task 3). Neurobehavioral disruptions are evident and associated neurochemical and histopathological changes combination injuries are being evaluated in ongoing experiments (figs 4-8 in Supporting Data section).

Task 3: Combine BOP and the selected impact acceleration insult at multiple combined severities, and evaluate the histopathological, physiological, and neurobehavioral outcomes relative to those seen following each insult alone. Establish combined injury conditions to produce mTBI.

Combinations of BOP and weight drops of varied heights have been completed using all 3 weight drop approaches described above (task 2). Three BOP severities were paired with 3 weight drop severities. To this point they have been most successful using discs affixed to the rats' skulls and discs held in place by a light weight Mylar headpiece. For the former, to eliminate possible interference by the discs of the biomechanical effects of BOP on the cranium, discs were secured to the skull immediately after BOP exposure, which imposed a 10 min separation between the BOP and impact-acceleration insults to the brain. Despite this temporal constraint, we documented persistent neurobehavioral disruptions with combined insults. Notably, in rats subjected to weight drops from 125 cm onto skull discs 10 min after BOP, there was a persistent neurological deficit seen on the rotating pole that was not observed in rats subjected to either insult alone, or to BOP combined with weight drops from a lesser height (fig 4 in Supporting Data section). These findings are striking and are entirely consistent with the primary hypothesis of the project, namely that the TBI resulting from these combined insults is fundamentally different from that seen from either insult alone. Initial results from ongoing neurochemical assessments reveal a similar interaction or interplay between insults such that the pathophysiological DNA fragmentation and inflammatory disruptions produced by combined injuries exceed those produce by either alone (figs 5 & 6 in Supporting Data section).

Task 4: Using a mach stem wedge equipped with a high velocity piston impactor, instantaneously combine impact acceleration with BOP within the shock tube to produce and evaluate the concomitant combined effects of primary and tertiary blast relative to those seen following each insult alone. Establish a mild injury severity based upon loss of consciousness (LOC), histopathology, and neurological and neurobehavioral outcomes.

Task 4 progress: Not started.

KEY RESEARCH ACCOMPLISHMENTS:

- Shock tube BOP exposure conditions have been characterized and refined to create a high fidelity simulation of blast TBI.
- Neurobehavioral, neuropathological, and neurochemical consequences of shock tube BOP exposures of varied intensities have been described and are ongoing.
- Neurobehavioral, neuropathological, and neurochemical consequences of weight drop-induced impact acceleration of varied intensities, alone and in combination with shock tube BOP exposures, have been described and are ongoing.
- EEG recordings have been initiated to distinguish electrophysiological consequences of individual and combined blast- and weight drop-induced brain insults.

REPORTABLE OUTCOMES:

Based upon work supported by this award, funding was sought through research pre-proposals and proposals submitted to the CDMRP and DMRP, which included:

- Imaging biomarkers for mild blast-induced traumatic brain injury
- Blast-induced acceleration in a shock tube: distinguishing primary and tertiary blast injury mechanisms in rat TBI
- Roles of polyunsaturated fatty acids in traumatic brain injury vulnerabilities and resilience: evaluation of salutary effects of DHA supplementation using neurolipidomics and functional outcome assessments
- Diagnostic and Therapeutic Targeting of Neuroinflammation in Blast TBI
- Novel nitroxide-based therapy to optimize 100% oxygen use in critical traumatic brain injury resuscitation and transport

CONCLUSION:

Results to date are consistent with the hypothesis that BOP generates a closely-associated insult to the brain (and other organs as well) and interactively compromises the brain's resilience and exacerbates the pathophysiological effects of other injury modalities such as impact acceleration (i.e. tertiary injury). With continued refinement, under carefully controlled experimental conditions the combined biomechanical perturbations of the brain that yield blast-induced mild TBI in injured warfighters can be recreated with reasonable fidelity to reproduce characteristic sequelae of blast-induced mild TBI. The end product model will provide an invaluable tool to define underlying neurobiological mechanisms and rationally establish effective countermeasures to lessen short-term impairments (e.g. return-to-duty) as well as chronic debilitation (e.g. chronic traumatic encephalopathy).

REFERENCES:

Mac Donald CL, Johnson AM, Cooper D, Nelson EC, Werner NJ, Shimony JS, Snyder AZ, Raichle ME, Witherow JR, Fang R, Flaherty SF, Brody DL. Detection of blast-related traumatic brain injury in U.S. military personnel. N Engl J Med. 2011 Jun 2;364(22):2091-100.

Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J Neurosurg. 1994 Feb;80(2):291-300.

APPENDICES:
None

SUPPORTING DATA:
BOP exposure of isoflurane-anesthetized rats occurs in a shock tube (fig 1). Rats are suspended in coarse mesh netting in a gauged holder that records incident and side-on pressures. Accelerometers positioned on the heads and trunks of experimental subjects have been used to quantify acceleration associated with blast flow conditions. Under these carefully controlled experimental conditions, blast-induced biomechanical perturbations can be recreated with reasonable fidelity. Immediately after BOP exposure, rats are removed from the shock tube and subjected to weight drop-induced impact acceleration head injury (task 3). In addition to 500g weights dropped 100-200 cm through a cylinder onto a 10 mm stainless steel disc affixed to the skull (as described by Marmarou et al. (1994), we have used an alternative novel device (fig 2) in which weight is dropped onto a readily applied and removed gauged helmet. Unfortunately, we encountered difficulty producing head injuries with this procedure, prompting a third approach in which a 10 mm stainless steel disc is rapidly positioned on the scalp using a light weight Mylar head piece for a surgery-free weight drop that minimizes time constraints on dual injuries.

Fig 1. Shock tube, gauged holder, pressure and acceleration tracings when positioned 2.5 within the mouth of the shock tube.

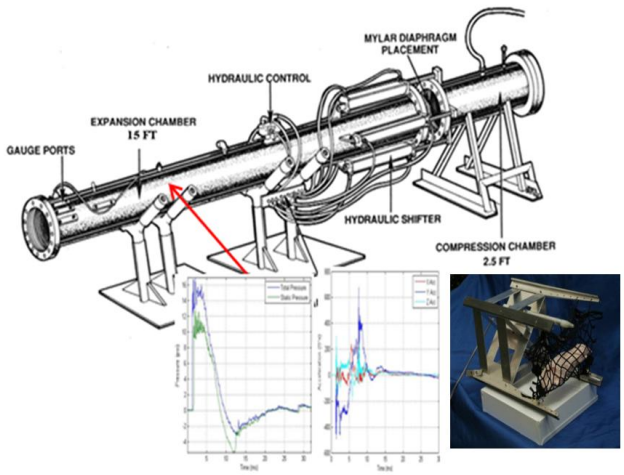
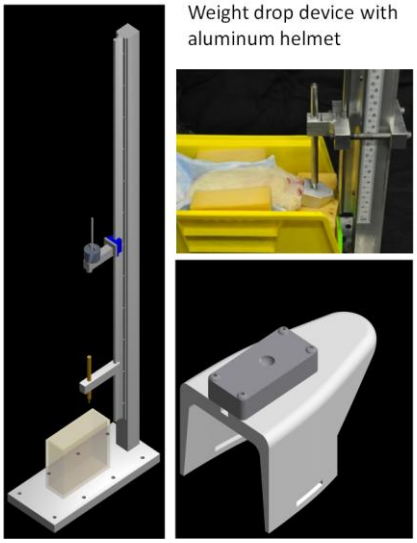


Fig 2. Weight drop injuries.



Among the functional neurobehavioral assessments that are used to evaluate these rats, a rotating pole is used to assess neuromotor impairments. At varied times postinjury, pretrained rats traverse a rotating pole and are graded using a scoring scheme that incorporates balance (1 point for not falling; 0 points for a fall), velocity (distance on the pole covered/time) and distance completed (1 point for a complete run, 0.75 for a fall at the ¾

Fig 3. Rats traverse a pole rotating at 4.0 rpm

mark, 0.5 for a fall in the middle and 0 points for a fall at the beginning). On each test day, each rat is given three trials. The two highest score runs are averaged as the score for that rat on that day. BOP (11-21 psi) and weight drop (100-200 cm) each produced severity-dependent impairments that are evident with the rotating pole. When BOP and weight drop are combined, we have established that following a 12 psi BOP a 125 cm weight drop produces lasting deficits that are not seen following either BOP or weight drop alone or with less severe weight drop impacts (fig 4). This combination of BOP and weight drop insults also produces DNA fragmentation and reactive oxygen species production that are not seen following either insult alone (fig 5). Similarly, immunohistochemical assessments reveal greater glial fibrillary acidic protein (GFAP) and IBa1 immunoreactivity in rats subjected to combined injuries than are seen following either injury alone (figs 6 and 7). Primary antibodies of anti-Iba-1 (WAKO Pure Chemical Industries, Osaka, Japan) and anti-GFAP (Cell signaling, Danvers, MA) were used in the immune-double staining for activated microglia/ macrophages and astrocytes, respectively. Expression of Iba1 (red) and GFAP (green) was greatly increased in cortex, striatum and corpus callosum 15 days after combined BOP and weight drop injuries. Nuclei in the right panel were stained blue by Hoechst reagent (Invitrogen, Grand Island, NY).

Fig 4. Rotating pole scores following BOP, weight drop, and combined injuries.

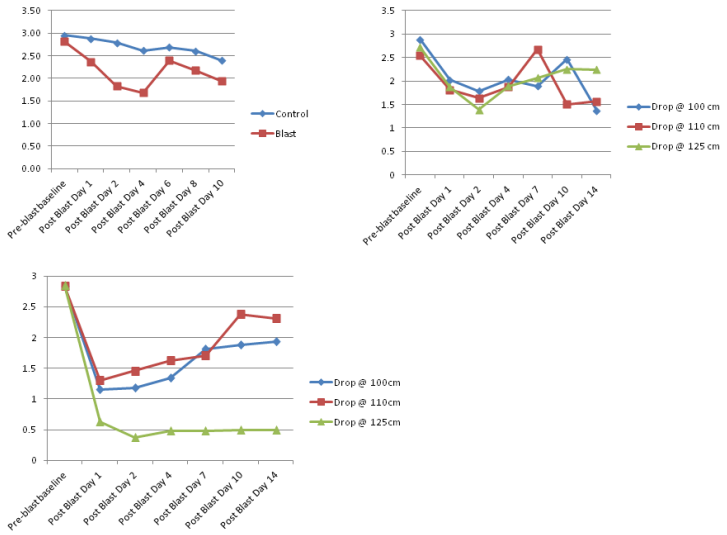
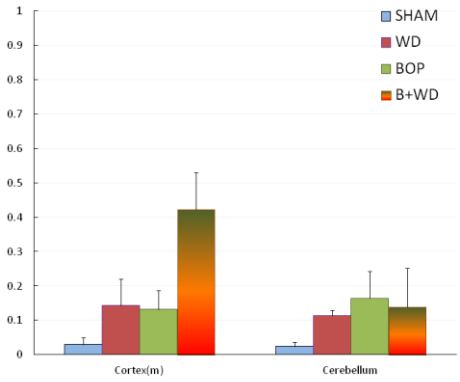


Fig 5. DNA fragmentation after BOP, weight drop, and combined injuries.



The hallmark neuropathological feature in rat brains following BOP exposure is widespread fiber degeneration that is most prominent in cerebellum, optic tracts, and external capsule (fig 7). Similar injuries are evident in weight drop-injured brains and are accompanied by additional degeneration in the pyramidal decussation, paraolivary nucleus, and cerebral cortex, which are typically much less affected by BOP. Although these assessments are incomplete and ongoing, neuropathological scoring completed to date suggests higher levels of injury following combined insults.

Fig 6a. Fluorescent immunohistochemistry for activated microglia.

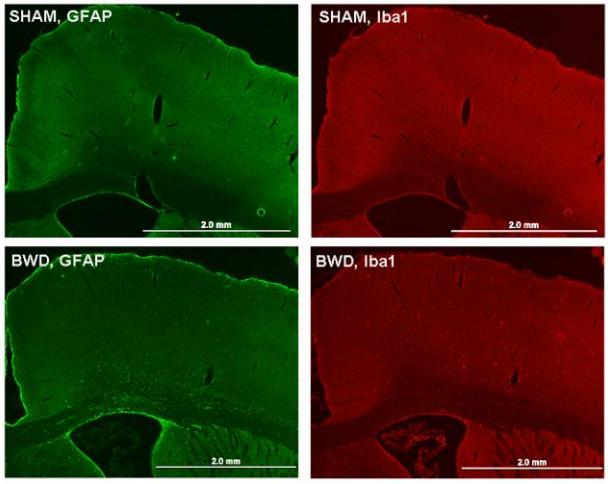


Fig 7a.

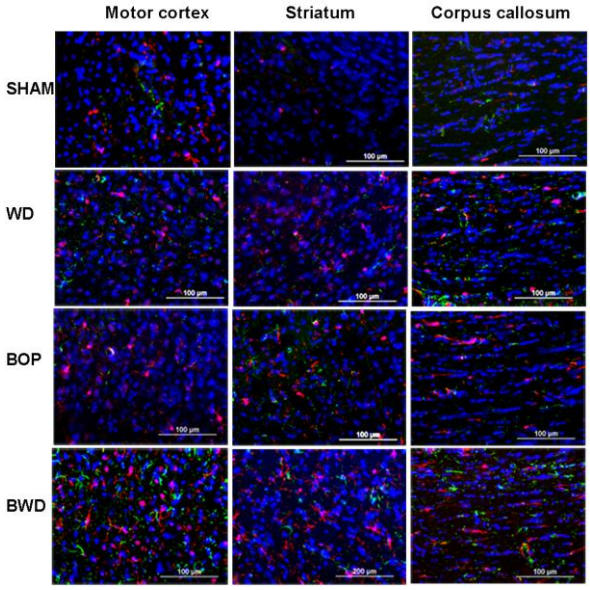


Fig 7b.

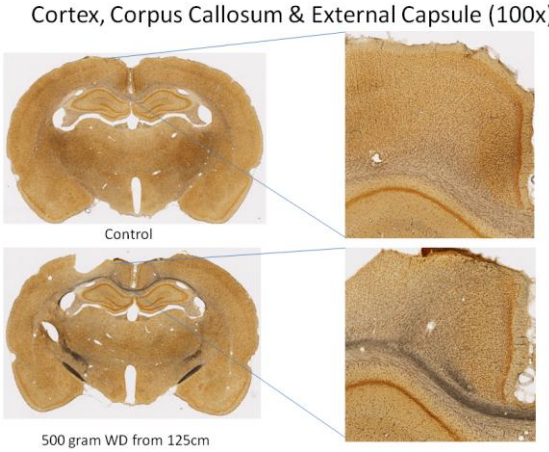


Fig 7c.

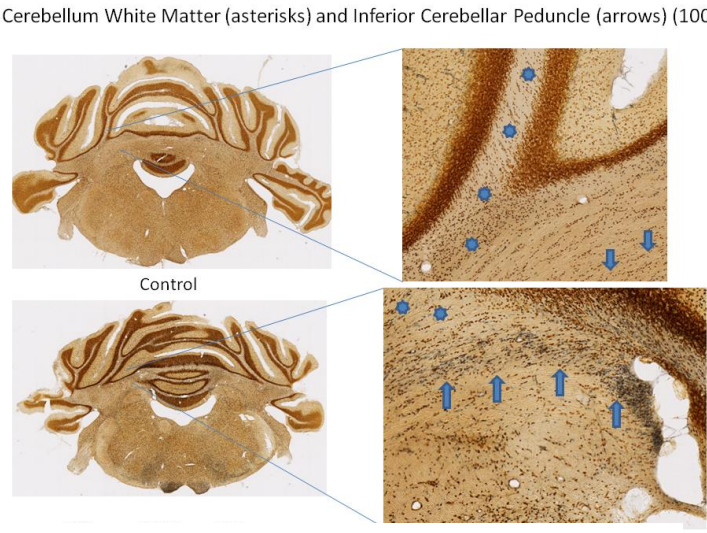
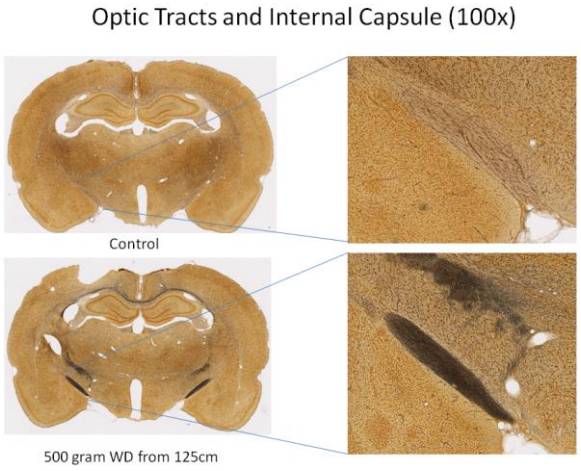
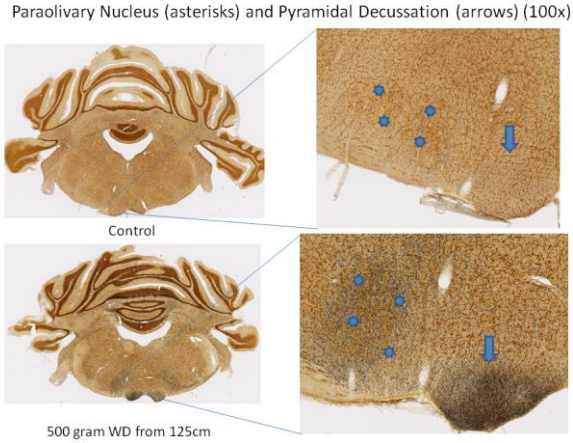


Fig 7d.





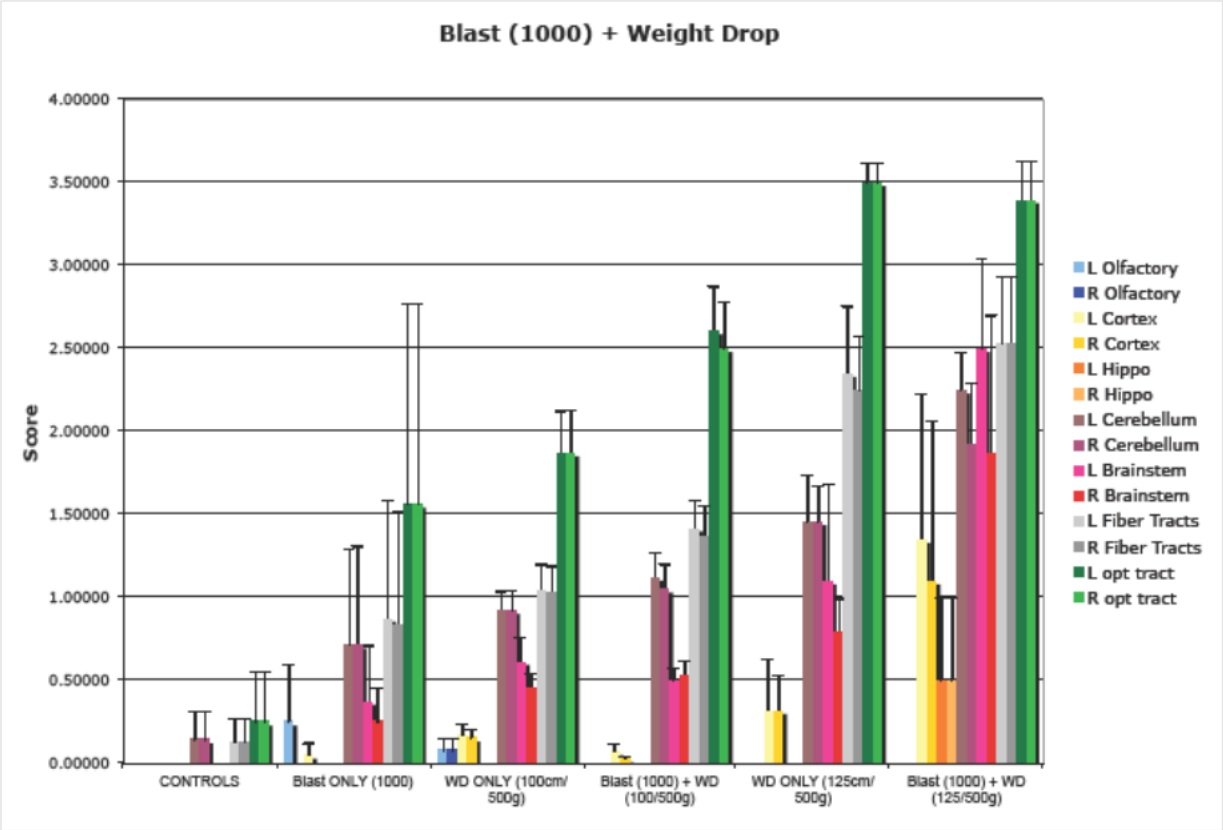

Blast (1000) + Weight Drop
Score
4.00000
3.50000
3.00000
2.50000
2.00000
1.50000
1.00000
0.50000
0.00000
CONTROLS
Blast ONLY (1000)
WD ONLY (100cm/ 500g)
Blast (1000) + WD (100/500g)
WD ONLY (125cm/ 500g)
Blast (1000) + WD (125/500g)
L Olfactory
R Olfactory
L Cortex
R Cortex
L Hippo
R Hippo
L Cerebellum
R Cerebellum
L Brainstem
R Brainstem
L Fiber Tracts
R Fiber Tracts
L opt tract
R opt tract

Fig 8.